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Genetics

A *GAL10–CYC1* hybrid yeast promoter identifies the *GAL4* regulatory region as an upstream site

(yeast promoter structure/galactose induction/glucose repression/upstream activation sites/inducer exclusion)

LEONARD GUARENTE*, R. ROGERS YOCUM†, AND PAULA GIFFORD*

*Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139; and †Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, Massachusetts 02138

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ABSTRACT **We have identified the promoter region of the *GAL10* gene (whose product is UDP-galactose epimerase) of *Saccharomyces cerevisiae*; this promoter mediates galactose induction of transcription in conjunction with the product of the *GAL4* regulatory gene. This identification was achieved by excising a 365-base-pair fragment of *GAL10* leader DNA with a *GAL10* proximal endpoint greater than 100 base pairs upstream of the transcriptional start site and substituting it in place of the upstream activation site of the *CYC1* (iso-1-cytochrome *c*) promoter [Guarente, L. & Ptashne, M. (1981) *Proc. Natl. Acad. Sci. USA* 78, 2199–2203]. The hybrid promoter is composed of DNA encoding *CYC1* mRNA start sites and the *GAL* segment upstream of these sites. This promoter is regulated in a manner analogous to *GAL10*; i.e., it is induced by galactose and responds to mutations in the *GAL4* and *GAL80* regulatory loci. The activity of the hybrid promoter requires sequences in the region of the *CYC1* mRNA start sites but does not require a precise spacing between these sequences and the *GAL* segment. The transposed *GAL* segment appears not to contain sequences that mediate glucose repression. Thus, the picture of the *GAL10* promoter that emerges is one of an upstream activation site that responds to the *GAL4* product plus galactose, and a region of transcription initiation that may contain sequences that mediate glucose repression. Experiments employing strains inducible (*GAL80*) or constitutive (*gal80*) for *GAL10* expression indicate that an additional component of glucose repression is inducer exclusion.**

Expression of prokaryotic genes or eukaryotic genes transcribed by RNA polymerase II is regulated by DNA sequences that lie upstream of coding sequences. In the cases of the simian virus 40 early region (1, 2), the sea urchin histone H2A gene (3), or the yeast *CYC1* gene (refs. 4 and 5) (unpublished data), these regulatory sequences lie in two regions, one in close proximity to where transcription initiates, and the other upstream of the initiation region. The *CYC1* gene, in particular, contains an upstream activation site (UAS_C) about 250 base pairs upstream of the startpoint of transcription that enhances expression about 50-fold (4).

To further study the role of UAS regions, we have probed whether yeast genes other than *CYC1* contain such sites. The focus of this report is the *GAL10* gene of *Saccharomyces cerevisiae*. The product of *GAL10* (UDP-galactose epimerase), along with the products of *GAL1* (galactokinase) and *GAL7* (galactose-1-phosphate uridylyltransferase), forms the pathway for utilization of galactose as carbon source in *S. cerevisiae*. These three genes are closely linked in a cluster on chromosome II (6, 7) and are coordinately induced about 1,000-fold at the level of transcription by growth on galactose (8–10). This coordinate control is exercised by the constitutively synthesized protein products of the *GAL4* and *GAL80* genes, which are linked neither to each other nor to the structural gene cluster (11–13). The *GAL4* protein is a positive regulator required for galactose induction, and the *GAL80* protein is a negative regulator thought to inhibit the action of *GAL4* in the absence of inducer, possibly by a protein–protein interaction (14). In the presence of galactose, the *GAL4* product is released from inhibition by *GAL80* and activates transcription of *GAL1*, *GAL7*, and *GAL10*, possibly by binding to DNA sequences at or near the 5′ ends of the three genes. However, the details of the mechanism of action of the *GAL4* and *GAL80* proteins remain to be determined.

In addition to the *GAL4* and *GAL80* regulation, *GAL1*, *-7*, and *-10* are subject to glucose repression. Growth in galactose plus glucose leads to strong repression in the levels of the galactose utilization enzymes compared to growth in galactose alone (15, 16). Nonfermentable carbon sources such as glycerol or lactate do not show this repression. Transcription of the *CYC1* gene is also under glucose repression (17),‡ as is transcription of genes encoding invertase and maltase (18, 19).

The region of chromosome II containing *GAL1*, *-7*, and *-10* has been cloned and the transcribed regions have been identified (6, 7). The structure of this region is summarized in Fig. 1. Each gene encodes a distinct mRNA. *GAL1* and *GAL10* are divergently transcribed and there is a 650-base-pair region between the two coding sequences. *GAL7* is transcribed in the same direction as *GAL10* and lies past the 3′ end of that gene. The DNA sequences that mediate activation by the *GAL4* product presumably lie in the region between *GAL1* and *GAL10* and in the region between the 3′ end of *GAL10* and the 5′ end of *GAL7*.

In this report we describe a demarcation of the *GAL10* control region. Specifically, we have identified a region more than 130 base pairs upstream of the *GAL10* transcriptional start site that mediates *GAL4* activation of transcription. When this region is substituted in place of the *CYC1* UAS, it confers galactose-specific control on *CYC1* transcription. Further, the region appears not to contain the sequences responsible for glucose repression of *GAL10* transcription. Similarities between the structures of the *GAL10* and *CYC1* control regions are discussed.

MATERIALS AND METHODS

Yeast Strains. BWG2-9A (*α, his4-519, ade, ura3-52, gal4*) was obtained by mating BWG1-7A (*a, leu2-3, leu2-112, his4-519, ade1-100, ura3-52*) with 279-1A (*α, gal4, trp1, ade6*) (a gift

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Abbreviations: UAS, upstream activation site; Amp^R, ampicillin resistance.

‡ Faye *et al.* (5) have found that derepressed *CYC1* mRNA initiates at seven sites within a region 90 to 30 base pairs upstream of the ATG. We have assigned the most upstream of these start sites as +1.

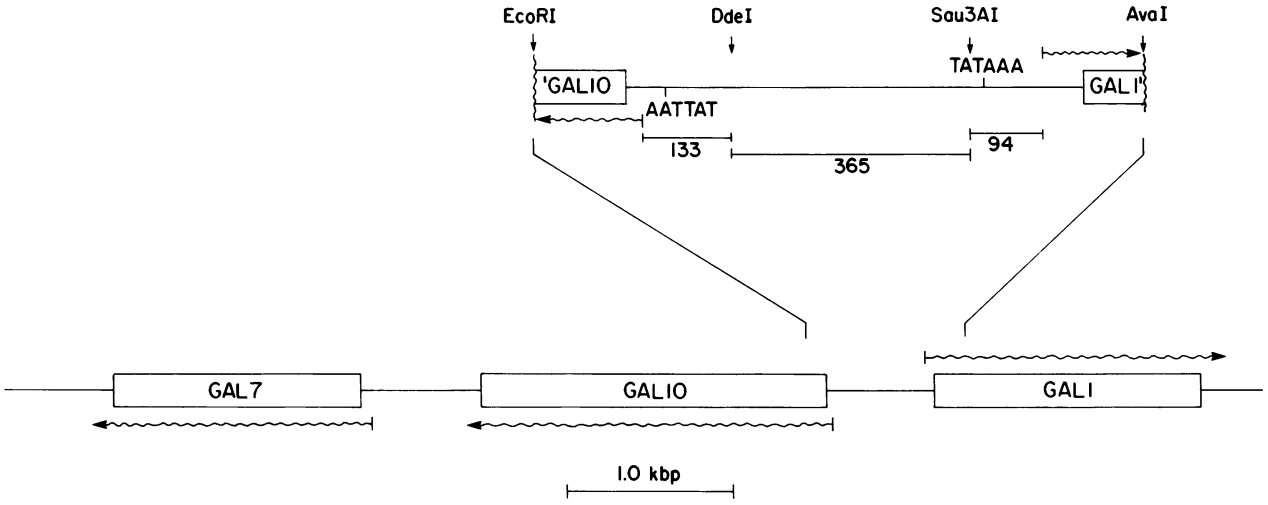

FIG. 1. *GAL7*, *-10*, and *-1* region of the yeast chromosome. Coding sequences are boxed and transcripts are indicated by wavy arrows (6, 7). The region between *GAL10* and *GAL1* is blown up. The *Sau*3A–*Dde* I segment that was excised from the gene cluster is 365 base pairs long (unpublished data) and its extremes lie 133 and 94 base pairs upstream of *GAL10* and *GAL1* mRNA start sites (M. Johnston, personal communication). Also shown are T-A-T-A-box-like sequences that lie upstream of the mRNA start sites (unpublished data) and *Eco*RI and *Ava* I restriction sites that lie in the *GAL10*, *-1* coding sequences. kbp, Kilobase pairs.

of Jim Yarger). Galactose nonutilization (Gal^-) and uracil auxotrophy (Ura^-) segregated 2:2 in the cross, and a Gal^-, Ura^- segregant was isolated.

LGI-ID (α, *ade*, *ura3-52*) was obtained by mating BWG2-9A with Sc106 (*a*, *leu1*, *trp1*, *gal80*) (a gift from Jim Hopper). Gal^- and Ura^- segregated 2:2 in the cross. *GAL4* segregants were screened for their *GAL80* allele by introducing pLGSD5 or RY123 and screening for whether β-galactosidase production was inducible by galactose (*GAL80*) or constitutive (*gal80*).

LGI-2D (*trp1*, *ura3-52*, *gal80*) was obtained from the same cross as LGI-ID.

Plasmids. Procedures for isolating and manipulating nucleic acids were as described (20).

pLGΔ-292 contains *CYC1* DNA out to position -292 and four base pairs of *CYC1*-coding DNA fused to *Escherichia coli lacZ*. The DNA encodes the mRNA start sites and, at -250, the *CYC1* UAS. The other features of this and the other plasmids are ampicillin resistance (Amp^R), *URA3*$^+$, and origins of replication from ColE1 and the yeast 2-μm circle (Fig. 2). pLGSD5 is identical to pLGΔ-292 except that DNA between the *Xho* I site at -158 and the *Sma* I site at -292 has been replaced with *GAL* DNA (see below). pRy123 contains no *CYC1* DNA but a fusion of *lacZ* to *GAL10* coding DNA. The fusion contains 46 codons of *GAL10* (through an *Eco*RI restriction site) and is preceded by the *GAL10-1* intergeneic region and a portion of *GAL1* (through the *Ava* I site). The structure of the remainder of the plasmid is identical to that of the others.

Construction of pLGSD5. pSc4816, a plasmid containing the *GAL10-1* intergeneic region (obtained from T. St. John) was digested with *Sau*3A and *Dde* I and the 365-base-pair intergeneic fragment (Fig. 1) was isolated. The ends of the fragment were rendered flush by DNA polymerase and the fragment was inserted into a backbone prepared as follows. pLG-292 was digested with *Xho* I, the ends were rendered flush by DNA polymerase, and a *Sal* I linker was inserted to give Δ-292S. This insertion regenerates *Xho* I sites on both sides of the *Sal* I linker. The plasmid so derived was then digested with *Sal* I and *Sma* I, the ends were rendered flush by DNA polymerase, and the GAL fragment was inserted (Fig. 2). The orientation of the SD5 insert was determined by mapping the position in the recombinant of the *Alu* I site, which lies asymmetrically on the fragment. Note that pLGSD5 contains a single *Xho* I site, at the *GAL–CYC1* junction.

Insertion of Deletion Fragments from pLGΔ-292 into pLGSD5. DNA fragments with deletions extending from the *Sal* I site (at -158) of pLGΔ-292S to -22, $+23$, or $+54$ retain *Xho* I sites at their deletion endpoints. These fragments were constructed by ligating fragments extending from the *Bam*HI site at the *CYC1–lacZ* junction but to -22, $+23$, or $+54$ into a backbone of pLGΔ-292S extending from the *Bam*HI site to a filled-in *Sal* I site. The ends at -22 and $+23$ correspond to *Mbo* II and *Ava* II sites that were rendered flush by DNA polymerase, and the $+54$ endpoint was generated by *Bal* 31 exo-

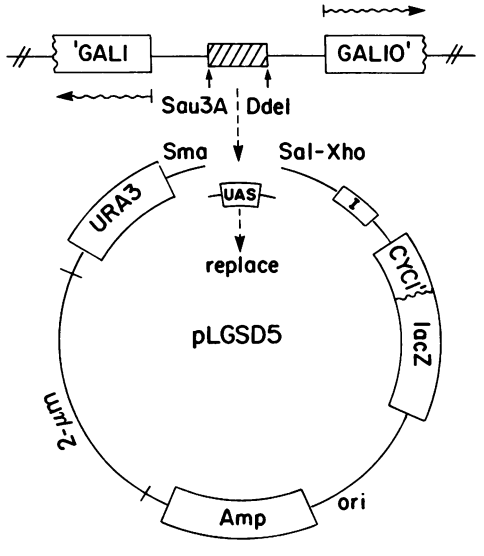

FIG. 2. Construction and structure of pLGSD5. The *Sau*3A–*Dde* I fragment excised from pSC4816 (note that the orientation of *GAL1* and *GAL10* have been reversed from Fig. 1) was rendered flush ended and inserted into a pLGΔ-292S backbone extending from a *Sal* I end that had been rendered flush to a *Sma* I end. This insertion substitutes the *GAL* segment for UAS_G (ref. 4; unpublished data). Other features of pLGSD5 are the *CYC1* I region, which encodes the T-A-T-A box and mRNA start sites, a *CYC1–lacZ* fused gene (4), origins of replication for *E. coli* (*ori*) or yeast (2-μm), and markers selectable in *E. coli* (Amp^R) and yeast (*URA3*). The wavy arrow indicates transcripts.

nuclease. The fragments extending from −22, +23, or +54 to the *Bam*HI site at the *CYC1–lacZ* junction were excised, purified, and inserted into a pLGSD5 backbone extending from the *Xho* I site to the *Bam*HI site.

Media and Assays. Cells were grown in minimal media (0.7% yeast nitrogen base plus required amino acids at 4 mg/ml) supplemented with 2% glucose, 2% galactose, or 2% glucose and 2% galactose.

β-Galactosidase assays were performed as described (4, 20). Briefly, cells were grown to OD_{600} = 1, spun down, resuspended in 1 ml of Z buffer (21), and made permeable by the addition of three drops $CHCl_3$ and two drops 0.1% sodium dodecyl sulfate and swirling on a Vortex mixer, and hydrolysis of *O*-nitrophenyl galactoside was measured at 28°C.

RESULTS

Substitution of the *CYC1* UAS with the *GAL10* Upstream Segment. A segment of DNA bounded by *Sau*3A and *Dde* I restriction sites was excised from the region between *GAL10* and *GAL1* (Fig. 1). The *Dde* I site lies 160 base pairs upstream of *GAL10* coding DNA, and the *Sau*3A site lies 160 nucleotides upstream of *GAL1* coding DNA (unpublished data). The *Sau*3A–*Dde* I fragment is 365 base pairs long. The mRNA startpoints for *GAL10* and *GAL1* lie 27 and 66 base pairs, respectively, upstream of coding DNA (M. Johnston, personal communication). Thus, the *Sau*3A–*Dde* I fragment contains neither the mRNA start sites for *GAL10* or *GAL1* nor any T-A-T-A box sequences that lie upstream of these start sites (unpublished data). The fragment was inserted into the *CYC1* leader DNA between the *Sma* I and *Xho* I restriction sites (Fig. 2). This generated a substitution of the *GAL* fragment for the UAS in the *CYC1* promoter region. The junction between the *GAL* fragment and *CYC1* DNA is at a *Xho* I site 158 base pairs upstream of the *CYC1* mRNA start.‡ The particular substitution we will focus on in this report, SD5, is oriented with the *GAL10* proximal end closer to *CYC1*.

The plasmid containing this substitution, pLGSD5, bore a fusion of *CYC1* coding DNA to *lacZ* (4) so that expression could be monitored by measuring levels of the *lacZ* product, β-galactosidase. The plasmid also contained markers that could be selected in *E. coli* (Amp^R) or yeast (*URA3*) and origins of replication that functioned in the respective organisms (from ColE1 and the 2-μm circle).

Expression of pLGSD5. Strains bearing various alleles of the *GAL4* and *GAL80* regulatory loci were transformed with pLGSD5, and regulation of expression was examined under a variety of physiological conditions (Fig. 3). Several pertinent results were obtained.

(*i*) Expression was strongly inducible by galactose (with a galactose-to-glucose ratio of about 1,000) in a *GAL4*, *GAL80* background. This is in agreement with previous measurements of the induction of *GAL10* mRNA (7). In contrast, expression driven by the *CYC1* UAS (UAS_C) was quite high in glucose and was only slightly derepressed (2.5-fold) by galactose.

(*ii*) Galactose induction of pLGSD5 expression did not occur

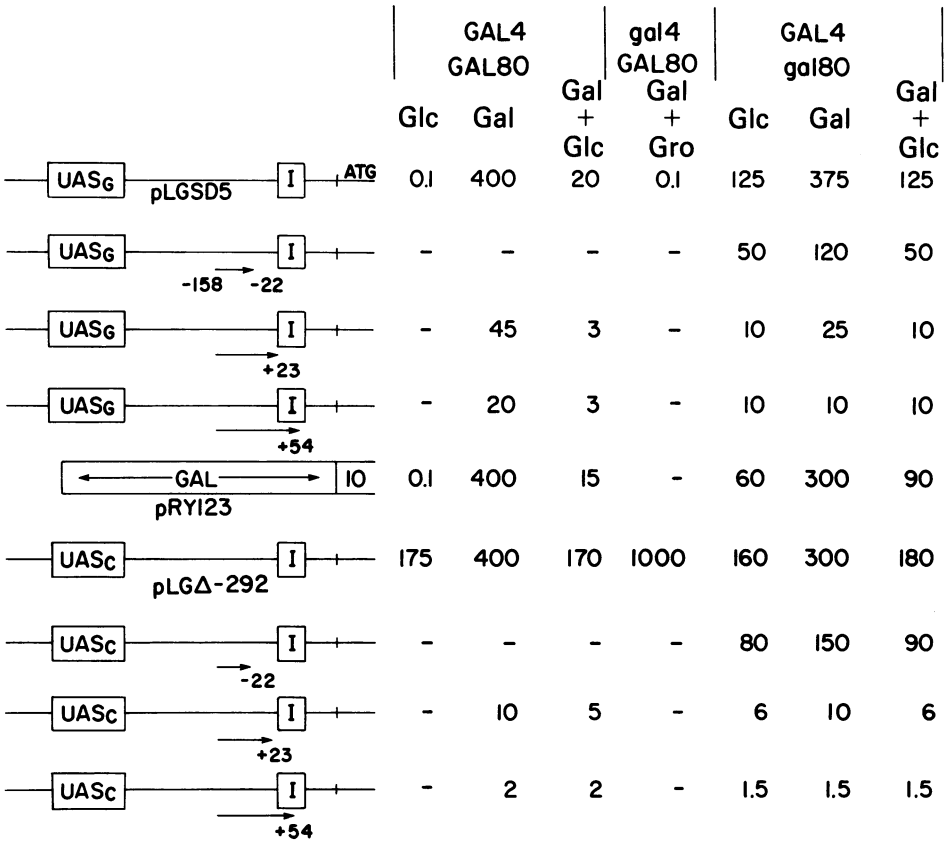

FIG. 3. Levels of β-galactosidase [units according to Miller (21)] driven by promoters with the indicated structures in strains with the indicated *GAL4* and *GAL80* alleles. Promoters contain either the SD5 UAS_G segment or the *CYC1* UAS_C in the upstream position. The initiation region (I) is from *CYC1* in all cases except pRY123, which contains an intact *GAL10* promoter. Deletions are indicated by the arrows and have the indicated endpoints (relative to the *CYC1* mRNA start sites‡). The yeast strains LG1-1D (*GAL4*, *GAL80*), BWG2-9A (*gal4*, *GAL80*), and LG1-2D (*GAL4*, *gal80*) are described in the text. Cells were grown in minimal media supplemented with 2% galactose (Gal), 2% glucose and 2% galactose (Gal + Glc), or 2% galactose, 2% (vol/vol) glycerol, 2% (vol/vol) ethanol, and aspartate at 40 μg/ml (Gal + Gro). β-Galactosidase was assayed as described in the text. Assays were done at least three times and the error was less than 10%.

in a *gal4* strain. Expression under control of the UAS_C was unaffected by the *GAL4* mutation.

(*iii*) pLGSD5 expression was constitutive in a *gal80* strain. Again, the UAS_C-activated expression was not affected by the mutation.

(*iv*) Galactose induction of SD5-derived expression was decreased by addition of glucose to galactose media. This effect is examined in greater detail in a subsequent section.

As a control, we examined the regulation of a direct fusion of the *GAL10* gene to *lacZ* in plasmid RY123 (Fig. 3). The pattern of regulation seen in this case was similar to that observed for pLGSD5. Thus, by substituting the UAS_C with the *GAL10* upstream segment, we have rendered *CYC1* regulation dependent upon the *GAL* regulatory loci. Presumably, the SD5 segment contains the site that mediates activation by the *GAL4* product (UAS_G).

UAS_G Expression Requires Sequences in the *CYC1* Initiation Region. Because the SD5 segment did not contain the sites of *GAL10* transcription initiation or the corresponding T-A-T-A box sequence, we considered it unlikely that transcription in pLGSD5 initiated in or close to the substitution. Rather, we suspected that the segment promoted initiation of transcripts with 5′ ends identical to *CYC1* transcripts. To obtain evidence that this was the case, we introduced a series of deletions into pLGSD5 extending from the *Xho* I site at the *GAL–CYC1* boundary toward the region of *CYC1* transcription initiation. These deletions were isolated in *cis* to the UAS_C and resulted in decreased expression as shown in Fig. 3.

A similar decrease in the induced levels of expression was observed when the deletions were *cis* to the UAS_G. In the constitutive *gal80* strain in minimal glucose medium, deletions extending from −158 to −22, +23, and +54 decreased expression to 40%, 8%, and 8%, respectively. Thus, UAS_G-activated expression requires sequences in the *CYC1* initiation region, as does UAS_C-activated expression.

For reasons that are not apparent, the +54 deletion caused a greater decrease in expression when *cis* to the UAS_C (to 1%) than when *cis* to the UAS_G (to 8%).

Deletions in the *CYC1* Initiation Region Alter the Glucose Response. We examined the glucose repression response of pLGSD5 in more detail. We found that expression was decreased to about 5% in a *GAL4*, *GAL80* strain background and to about 30% in a *GAL4*, *gal80* background (Fig. 3). The direct *GAL10–lacZ* fusion in pRY123 displayed a similar pattern of expression.

There were two promoter regions that could be responsible for the glucose response of pLGSD5. First, the UAS_G could contain the site that normally mediates the glucose response of *GAL10*. Second, sequences in the *CYC1* initiation region could be responsible for the reduction observed in pLGSD5.

To distinguish between the two possibilities, we examined the effects on repression of the deletions in the *CYC1* initiation region described in the previous section. Expression was determined in both a constitutive *gal80* strain, to avoid problems of inducer (galactose) entry into the cells, and in the inducible *GAL80* strain (Fig. 3). In the *gal80* strain, derepression in media without glucose was observed when the plasmid bore the deletion ending at +23 but was abolished by the deletion extending to +54. This effect was also seen when the deletions were *cis* to the UAS_C. In the *GAL80* strain, the +54 deletion reduced the magnitude of the glucose response (from 20-fold to 7-fold) but did not eliminate the effect. The *GAL80* allele had little effect on UAS_C-activated expression.

DISCUSSION

In this report we have demarcated the control region of the *GAL10* gene of *S. cerevisiae*. We have shown that a region 132 to 496 base pairs upstream of the start of *GAL10* transcription (M. Johnston, personal communication) contains the site responsible for transcriptional activation by the *GAL4* protein plus galactose. To do this, we excised the above region on a fragment bounded by *Dde* I and *Sau3A* restriction sites from a region that lies between the divergently transcribed *GAL1–GAL10* cluster (Figs. 1 and 2). The fragment was then inserted into a plasmid containing a *CYC1–lacZ* fused gene. This resulted in a substitution of a region of the *CYC1* promoter, the upstream activation site (UAS_C), with the *GAL10* segment. The *GAL10* proximal end of one such substitution, SD5, lay proximal to *CYC1–lacZ* in the resulting plasmid, pLGSD5. This substitution conferred galactose-specific regulation, dependent upon *GAL4* and *GAL80*, on *CYC1* transcription. When activated by its own UAS, *CYC1* transcription showed no such regulation.

We believe that the *GAL10* UAS (UAS_G) in pLGSD5 promotes initiation at the normal *CYC1* start sites for several reasons. First, the segment does not encode the *GAL10* (or *GAL1*)

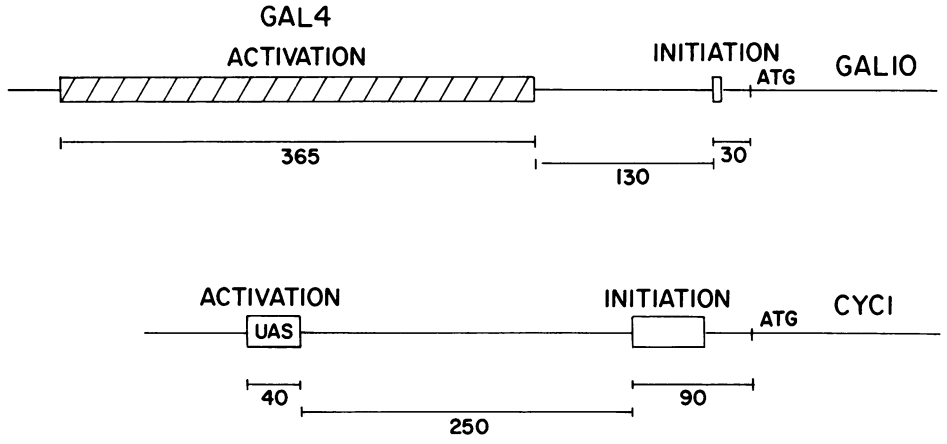

FIG. 4. The *GAL10* and *CYC1* promoter regions are drawn to scale for comparison. The region of *GAL10* leader DNA that contains the *GAL4* activation site lies somewhere in the indicated 365-base-pair fragment, and the transcription initiation region is from M. Johnston (personal communication). The distances in base pairs from the activation site to the initiation region, 5′ border, and from that border to the ATG are indicated. The 50-base-pair *CYC1* initiation region contains multiple mRNA start sites (5), the most upstream of which is 90 base pairs from the ATG. The distance of this start site from the activation site and the size of the activation site are from unpublished data.

mRNA start sites or T-A-T-A box sequences. Second, if transcription were to initiate in or near the *GAL* substitution, an mRNA with a long leader containing several AUG triplets would be encoded. These triplets would all be out of phase with the *CYC1–lacZ* fused gene. Because translation of yeast mRNA is thought to initiate at the AUG triplet closest to the 5′ end of the mRNA (22), the above mRNA would not be expected to be translated to give a functional protein. Third, and most importantly, deletions in the region of *CYC1* transcription initiation, when recombined *cis* to the UAS_G, decreased expression substantially (Fig. 3). The same deletions also decreased expression when *cis* to the UAS_C. Thus, sequences in the *CYC1* initiation region (between −22 and +23) promote expression whether it is activated by the UAS_G or the UAS_C.

Apparently, a precise spacing between the UAS_G and the startpoint of transcription is not required. The separation between the *Dde* I site used to excise the *GAL* segment and the *GAL10* transcriptional start is about 130 base pairs (M. Johnston, personal communication). When substituted in place of the UAS_C, the terminus of the fragment lay 160 base pairs away from the most upstream of the *CYC1* mRNA start sites. Furthermore, a deletion that brought the UAS_G in close proximity to the *CYC1* mRNA start sites (up to −22) exerted only a 2-fold effect on expression. Similar flexibility has been observed in the spacing between the UAS_C and the transcriptional start sites (unpublished data).

Transcription of both *GAL1, -7, -10* and *CYC1* is decreased when glucose is added to galactose media. The *GAL–CYC1* hybrid promoter in pLGSD5 displayed a similar glucose effect. The effect was much greater in a *GAL80* strain (inducible) than in a *gal80* strain (constitutive) (20-fold versus 3-fold), suggesting that an inducer exclusion mechanism may operate in the *GAL80* strain. Exclusion of many carbon sources by glucose is very well established in *E. coli* (23–25). The 3-fold repression in the *gal80* strain was left intact by deletions extending from −158 to −22 or to +23 but was abolished by a deletion extending to +54 (Fig. 3). This result suggests that a site involved in repression may lie between +23 and +54. Because the above deletions also remove promoter sequences, it cannot be concluded that this site alone mediates repression of the intact promoter. It is clear, however, that the pLGSD5 derivative bearing the deletion extending to +54 is devoid of sequences that mediate repression. When this was introduced into the *GAL80* strain, the magnitude of the glucose effect was decreased from 20-fold to 7-fold. Thus we suggest that the effect seen in this strain is due in part to regulatory sequences in the initiation region and in part to inducer exclusion (7-fold).

We infer that the *GAL* segment in pLGSD5 does not carry sequences that mediate this repression. Thus, the site that mediates repression in pRY123 (which carries a *GAL10*–lacZ fusion) in the *gal80* strain must lie elsewhere. It is tempting to speculate that this site may reside in the region between the *GAL* segment and *GAL10*, plausibly in the *GAL10* initiation region.

Recent results suggest that the UAS_C activates transcription in response to heme (unpublished data). The structures of the *GAL10* and *CYC1* promoters, thus, look roughly similar (Fig. 4), upstream activation sites that respond to particular physiological stimuli, and regions that contain sites for transcription initiation and may contain sites for the more general glucose repression control.

Finally, pLGSD5 is of potential utility as a cloning vector [as detailed by Guarente (20)]. The insertion of a DNA segment encoding a gene and mRNA initiation site into the *Xho* I site of this plasmid should result in galactose-regulated expression of the encoded product. The ability to regulate levels of expression of cloned yeast genes may facilitate studies of the encoded proteins.

Note Added in Proof. Recent mapping of transcriptional initiation sites by S1 nuclease protection indicates that UAS_G- and UAS_C-activated transcripts initiate at precisely the same sites.

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